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Effect of gamma irradiation on cytokines released by platelets during storage

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ABSTRACT

Most published gamma irradiation studies are on the cytokines secreted by leucocytes than platelet cytokines. If cytokines secretion is suppressed by irradiation then it will be an important application of irradiation. To know this we planned to measure cytokines like Regulated upon activation normal T cells expressed and secreted (RANTES), platelet factor 4 (PF-4), Transforming growth factor βeta1 (TGF-β) and βeta Thromboglobulin (β TG) during the storage of irradiated (IR) and non-irradiated (NI) platelets. Ten platelet concentrate (PCs) were prepared using platelet rich plasma and buffy coat methods each and 10 by apheresis on Trima Cell separator. Each PC was transferred in a transfer bag and IR at about 25 Gy and stored at 22 °C in platelet agitator. Samples were taken from NI and IR bag each on 0 day, 3rd day and 5th day from supernatant for cytokine analysis. The samples were stored below −50 °C for cytokines assays using commercial ELISA kits. RANTES levels were in the range of 12–400 ng/ml on 0 day and increased to 108–800 ng/ml on 5th day in NI platelets and 104–800 ng/ml in IR platelet. PF4 increased from 0 day to 5th day showing levels 300–1500 ng/ml in both types of PC. β-TG range was 748–5258 ng/ml in NI and 878 –4638 ng/ml in IR platelets on 5th day. TGF-β1 increased up to 780–38431 pg/ml in NI and 461–50,000 pg/ml in IR PC. The study showed that comparison of cytokine levels during the storage in NI and IR platelets was not significant by Mann–Whitney U- test ($p > 0.05$). Significant increase was observed in these cytokine levels from 0 day to 3rd and 5th day in NI as well as IR samples by Wilcoxon Signed- Rank test ($p < 0.05$).

1. Introduction

Gamma irradiation of 25 Gy to cellular components of human blood is essential to prevent transfusion associated graft versus host disease (GVHD) (Pelsynski, Moroff, Luban, Taylor, & Quinonoes, 1994). Platelets do not contain nucleus and therefore host no DNA, platelets do inherit a genome in the form of messenger RNA. They contain numerous specialized organelles including α and dense granules, lysosomes,

Abbreviations: RANTES, Regulated upon activation normal T cells expressed and secreted; PF-4, platelet factor 4; TGF-β, Transforming growth factor βeta; β TG, βeta Thromboglobulin; PC, platelet concentrate; IR, irradiated; NI, non-irradiated; GVHD, graft versus host disease; PRP, platelet rich plasma; BC, Buffy coat; DAE, Department of atomic energy; BRNS, Board of research in nuclear sciences; BARC, Bhabha Atomic research centre; SDP, Single donor platelet.

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microperoxisomes and mitochondria (Mc Redmond et al., 2004). In a recent study of 5 Gy and 7 Gy irradiation damage mechanism on ultrastructures of platelets in rat cells there was profound destruction at the cell membrane because of the free radicals formed by ionizing irradiation. There was cytoplasmic outflow and organelle damage where the platelet granules were reduced markedly (Ji, Dong, & Chung, 2012). It is possible that if the granules are reduced the release of cytokines may also be suppressed by irradiation. If so then it will be an important application of irradiation. Therefore this study was undertaken and it will be interesting and important to know if there is any suppression of cytokine secretion by platelets after irradiation by measuring levels of cytokines like RANTES, PF4, TGF-β and β-TG in NI and IR platelets.

2. Material and methods

2.1. Sample collection

The study was approved by the institutional ethics committee. It was funded by the BRNS, (DAE). Gamma irradiator, BI 2000 was purchased from Board of radiation and isotope technology, (DAE), Mumbai and regulatory permissions were obtained from atomic energy regulatory board.

After selection of blood donor as per Drugs and cosmetics rules 1945, the blood was collected in triple or quadruple bags containing Citrate–Phosphate–Dextrose-A1 from donors attending blood donation camps and donating in-house (Saran, 2003). PCs were prepared within six hours of blood collection.

2.2. Preparation of PC

Two types of PC were prepared namely PRP-PC and BC-PC using two different principles for separation. Ten PRP-PC were prepared from triple bags by centrifuging the bags at 20–22 °C at light spin and then heavy spin. PC was left undisturbed for one hour, and then the platelets in plasma were resuspended by gently mixing. Platelets were stored at 20–22 °C in platelet incubator with agitator (Benson et al., 1996). BC method was employed for preparation of 10 PCs using 'top and bottom' bags on Optipress II component extractor of M/S Fenwal (Bharucha, Chiewsilp, & Bhasin, 2002). Ten SDP were prepared using cell separator Trima (Gambro Bct) and stored at 20–22 °C.

2.3. Irradiation

All PCs were divided into two parts. NI part was named as "A" and IR as "B". Part B was put in the sample chamber of blood irradiator BI-2000 in inverted position, so no tubing was protruded outside the sample chamber and irradiated at about 25 Gy, dose rate 6.9 Gy/min (irradiated for 3 min 37 s).Co-60 is the source of radiation in BI2000. Five ml PC sample of A and B parts was collected on 0, 3 and 5th day aseptically and after centrifugation, one ml aliquots were prepared and stored below −50 °C for measurement of cytokines. Before assay the samples were thawed at room temperature and centrifuged at 3000 rpm for 5 min. Cytokine analysis was done using ELISA kits for RANTES (Human CCL5-R & D Systems), PF-4 (Abcam), β-TG (USCAN Life Science Inc.) and TGF-β1 (eBioscence Inc.) on Fully automated ELISA system 'Freedom evo' (TECAN)

2.4. Statistical analysis

In this study Median, minimum, maximum and Non-parametric tests like Mann–Whitney and Wilcoxon signed rank test were used because the data obtained were not normally distributed (Mann & Whitney, 1947; Fay & Proschan, 2010).

3. Results

Total 30 units including 10 each of PRP-PC, BC-PC and SDP were taken in this study. RANTES, PF-4, β-TG, and TGF-β1 were measured from NI and IR PCs at 0, 3 and 5th days interval. The data of three types of PC were pooled for presentation of results.

Table 1 shows median levels of RANTES on different days of storage. The level on 0 day was below 70 ng/ml in half of the samples in both groups and five samples showed the values above 200 ng/ml in NI and seven in IR. The levels gradually increased from 0 day to fifth day and no sample was below 100 ng/ml and 50% of the samples were above 350 ng/ml reaching maximum to 800 ng/ml.

Table 2 shows median PF4 on different days of storage. On 0 day it was below 400 ng/ml in half of the samples in both groups and three samples showed the values above 700 ng/ml. The levels gradually increased from 0 day to fifth day and no sample was below 300 pg/ml and > than 50% of the samples were above 1300 ng/ml reaching maximum to 1500 ng/ml.

Table 3 shows median β-TG on different days of storage. β-TG level on 0 day was below 650 ng/ml in half of the samples in NI and ten in IR. Three samples showed the values around 2000 ng/ml in NI and around 1700 ng/ml in IR. The levels gradually increased from 0 day to fifth day and no sample was <700 ng/ml and >50% of the samples were above 2000 ng/ml reaching maximum to 5000 ng/ml.

Table 4 shows median TGF-β1 on different days of storage TGF-β1 level on 0 day in more than half of the samples in NI group were above 2000 pg/ml reaching maximum to 11,500 pg/ml. In IR group 20 samples were below 2000 pg/ml and maximum was 5400 pg/ml. The levels gradually increased from 0 day to fifth day and no sample was below 780 ng/ml and > than 50% of the samples were above 5000 pg/ml reaching maximum to 38,000 pg/ml in NI group and up to

Table 1 – Median RANTES levels in NI and IR PC on different days of storage.

Platelet concentrate	RANTES (ng/ml)		
	0 Day	3rd Day	5th Day
Non irradiated n = 30	67 (12–400)	319 (88–800)	382 (108–800)
Irradiated n = 30	88 (13–400)	296 (86–800)	383 (114–800)

Figures in the parenthesis show min–max values.

Table 2 – Median PF4 levels in NI and IR PC on different days of storage.

Platelet concentrate	PF4 (ng/ml)		
	0 Day	3rd Day	5th Day
Non irradiated n = 30	531 (128–750)	1189 (287–1500)	1304 (300–1500)
Irradiated n = 30	588 (135–750)	1247 (272–1500)	1305 (300–1500)

Figures in the parenthesis show min–max values.

Table 4 – Median TGF-β1 levels in NI and IR PC on different days of storage.

Platelet concentrate	TGF-β1 (pg/ml)		
	0 Day	3rd Day	5th Day
Non irradiated n = 30	1551 (390–11,508)	2919 (780–20,000)	5128 (780–38,431)
Irradiated n = 30	1023 (156–5409)	2018 (326–15,588)	8286 (461–50,000)

Figures in the parenthesis show min–max values.

31,000 pg/ml except one sample showing the value 50,000 pg/ml in IR group.

Statistical evaluation of cytokines in PC showed no significant difference in cytokines in NI and IR samples by Mann–Whitney U-test ($p > 0.05$). Significant increase was observed in these cytokine levels from 0 day to 3rd and 5th day in NI as well as IR samples by Wilcoxon Signed-Rank test ($p < 0.05$). Analysis on separating the types of PC showed that cytokine levels were significantly higher in BC-PC compared to PRP-PC and significantly high in SDP from 0 to 5th day in both NI and IR samples by Mann–Whitney test ($p < 0.05$).

4. Discussion

Gamma irradiation of cellular components to prevent TA-GVHD has been practiced since long time (Anderson et al., 1991). We irradiated the PCs at 25 Gy following the UK Blood transfusion published guidelines (Chapman et al., 1996) We investigated the accumulation of platelet derived cytokines RANTES, PF 4, TGF-β1 and β-TG in NI and IR platelets (Wadhwa et al., 2000).

The levels of RANTES in this study reached maximum to 800 ng/ml in IR and NI PCs which is comparable to the concentrations 668 ± 223 ng/ml reported by Klutter et al. to be associated with allergic symptoms (Klüter, Bubel, Kirchner, & Wilhelm, 1999). RANTES levels associated with allergic reactions have been reported to range from 200 to 1000 ng/ml (Turner, Sutherland, Wadhwa, & Cardigan, 2005; Shanwell, Falker, & Gulliksson, 2003; Wakamoto et al., 2003) Highest RANTES was found increased between day 1 and day 5 and others like TGF-β1 also increased which may be that after secretion from platelet, RANTES may trigger a positive feedback mechanism via the receptors of RANTES on surface of same or other platelet to secrete more protein and amplify response when aggregated. RANTES and TGF-β1 accumulate due to platelet injury and activation (Wadhwa et al., 2002). In this study the median RANTES level in NI PRP-PC are higher in SDP whereas in Fujihara study the RANTES levels in BCPC were slightly higher than in SDP (Fujihara, Ikebuchi, Wakamoto, & Sekiguchi, 1999). They performed gamma irradiation of PCs with 30 Gy whereas we used 25 Gy. Similarly Picker et al. showed the levels 248.7 ± 84.5 ng/ml in control and 239.7 ± 122.7 ng/ml in IR SDP (Picker, Steisel & Gathof, 2009). There is neither preventive effect nor adverse effect by irradiation because the values in IR and NI are similar.

PF4 median levels in PC were 531 ng/ml in NI on 0 day and 1304 ng/ml on 5th day. No significant difference was observed in NI and IR PCs. Shimizu et al. measured PF4 in WB and in PRP-PCs after different hours storage. In WB it was 6.3 ± 1.5 ng/ml immediately after phlebotomy, in PRP 26.8 ± 4.5 ng/ml and in PCs on agitator after resuspension it increased to 201 ng/ml. There was 5 percent increase in PF4 release after agitation and in storage than in PRP. Thus mechanical damage to platelets increases the release. It is also assumed that PC with high platelet count produces excess lactate resulting in fall in pH which cause alpha granular degranulation of PF4 (Shimizu, Ishikawa, Morishima, Fukuda, & Kato, 1985).

β-TG in our study increased from 249 ng/ml on 0 day to 5258 ng/ml on 5th day in NI which is not significantly different from IR PCs which reached 4698 ng/ml. Scott et al. showed that it is a measure of release reaction. They found spontaneous release of β-TG from platelets (Scott, Harris, & Bolton, 1983). Snyder et al. showed that centrifugation of platelets into button followed by resuspension damages the platelet. There is increased release of β-TG and PC should be kept for 60 min before resuspension (Snyder, Theodore, & Hezzey, 1982). Many times less attention is made to such practical issues and must have shown high levels. Snyder et al. found that during storage the percentage of β- TG release increased from 18.1% to 40.2%. Platelets after exposure to 10,000 rad dose did not stimulate platelet release (Snyder, Hezzey, Katz, & Bock, 1981). A survey of ionizing irradiation on blood and blood components published by International atomic energy agency (IAEA) states that irradiation of platelet at 100 Gy followed by 24 h storage did not induce an increased release of β-TG nor enhance discharge of lactate dehydrogenase (LDH) (IAEA 1997).

TGF-β in biological samples is often present in latent form and conversion to active form is necessary for estimation of total TGF-β content (Wadhwa et al., 2002; Wakefield et al., 1995). Samples were acid treated and neutralized to remove the latency associated peptide (LAP) prior to ELISA. Wadhwa's study included control for TGF-β where the values were mean

Table 3 – Median β-Thromboglobulin levels in NI and IR PC on different days of storage.

Platelet concentrate	β-Thromboglobulin (ng/ml)		
	0 Day	3rd Day	5th Day
Non irradiated n = 30	653 (249–2149)	2210 (683–4843)	3043 (748–5258)
Irradiated n = 30	966 (262–2089)	2004 (598–4118)	3453 (878–4698)

Figures in the parenthesis show min–max values.

13,000 (9000–27,000) pg/ml on day 1 and 43,000 (36,000–50,000 pg/ml) on day 5 in non filtered BC PCs which is comparable to our value of 38,431 pg/ml in NI and 50,000 pg/ml in IR PCs on 5th day. Apelseth et al. observed TGF-β 102,884 ± 17,007 pg/ml in untreated and 87,858 ± 15,148 pg/ml in IR (Apelseth, Hervig, Larsen, & Bruserud, 2006).

Thus gamma irradiation does not prevent the accumulation of cytokines. Platelet destruction and activation result in emptying of the contents of platelet granules into the supernatant of PC. Due to platelet activation cytokine synthesis occurs but cytokine synthesis will require optimal function of mitochondria like ATP and calcium uptake and unharmed mRNA to serve as template for synthesis. Thus irradiation does not have any effect on cytokine release. There may be release of preformed cytokines by destruction of cells. This shows that nuclear membranes and cell membranes are sensitive to irradiation but granules from where the cytokines are secreted have structure similar to obesity cells and are resistant to irradiation.

5. Conclusion

Platelet derived cytokines that is, RANTES, PF-4, TGF-β and β-TG were increased up to 5th day storage in NI and IR PCs prepared by PRP, BC methods and apheresis. There is no significant difference in cytokines in NI and IR PCs. Thus Gamma irradiation of platelet products neither enhanced nor prevented accumulation of these cytokines.

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